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## Solving the Polyketide Pigmentation Puzzle in *Fusarium solani*

*Linking biosynthetic genes to compounds*

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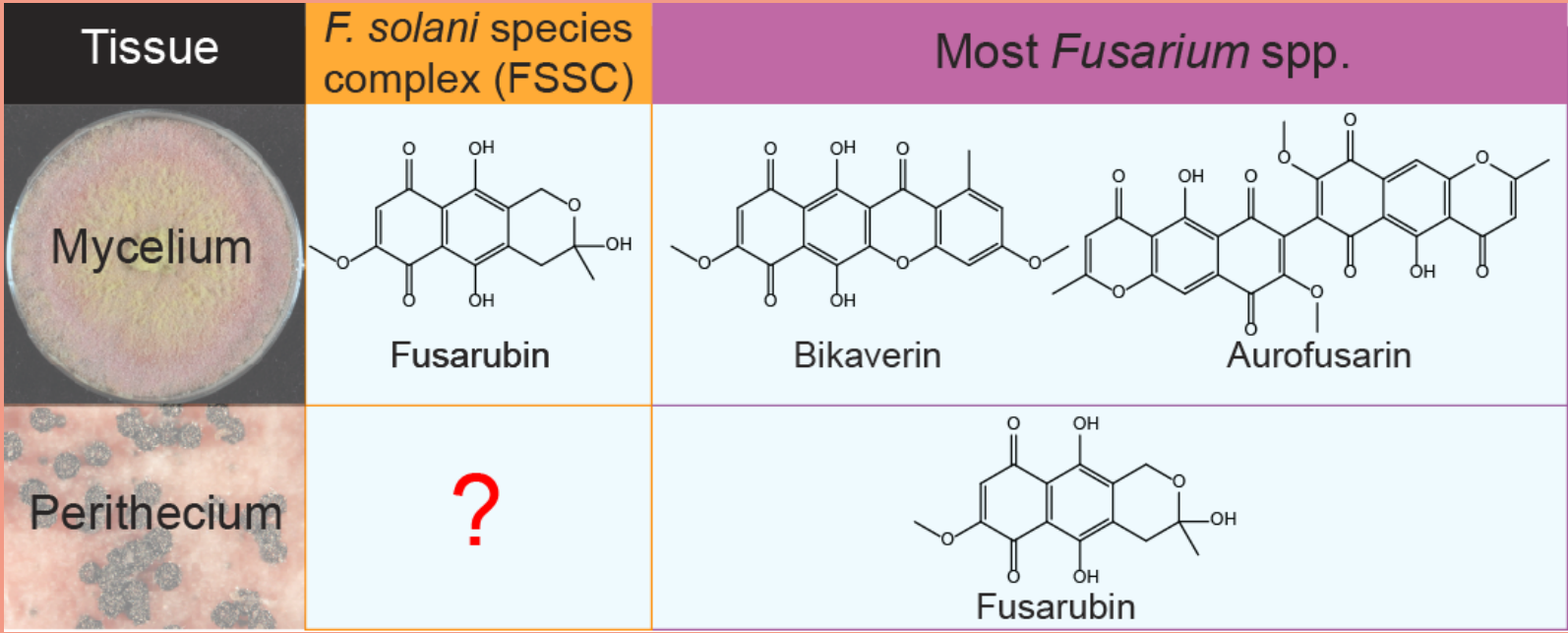
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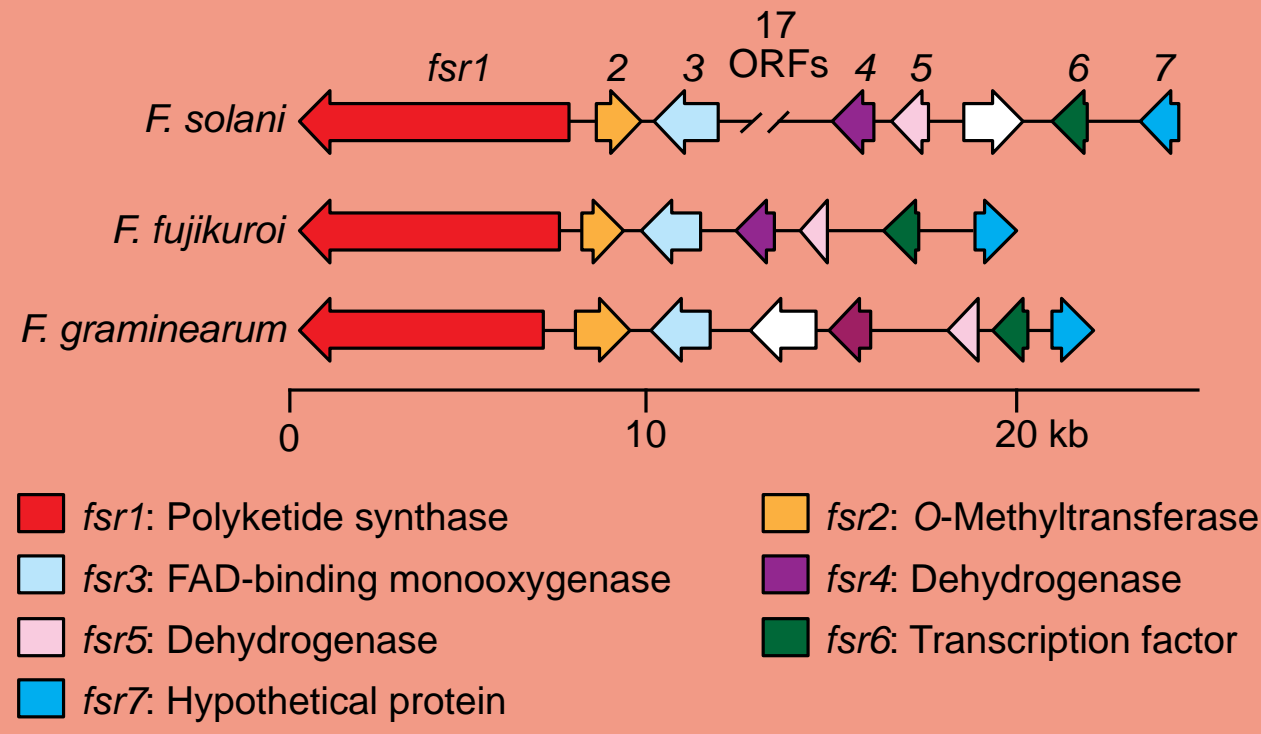
Introduction

*Fusarium* pigmentation is dictated by a set of two polyketide synthase (PKS) biosynthetic gene clusters (BGC) where one is expressed during mycelial growth and the other during perithecial development. In the vast majority of *Fusarium* species, perithecial pigmentation relies on the PKS3 (*fsr1*) cluster responsible for biosynthesis of the red and purple napthoquinone pigments fusarubin and bostrycoidin [1, 2]. However, the situation is different for members of the *Fusarium solani* species complex (FSSC), where mycelial pigmentation is controlled by the PKS3 cluster, while the clade-restricted PKS35 (*pksN*) [3] is responsible for perithecial pigmentation [4, 5], although no actual compounds(s) has ever been associated with the latter. In this study, we seek to associate the two *F. solani* polyketide pigmentation clusters to their respective compounds by an experimental approach. We aim to describe a set of novel pigment compounds – a category of secondary metabolites previously associated with structural diversity and biological function. We hope the techniques and methods applied can aid linking other fungal biosynthetic genes to their respective products in future studies.

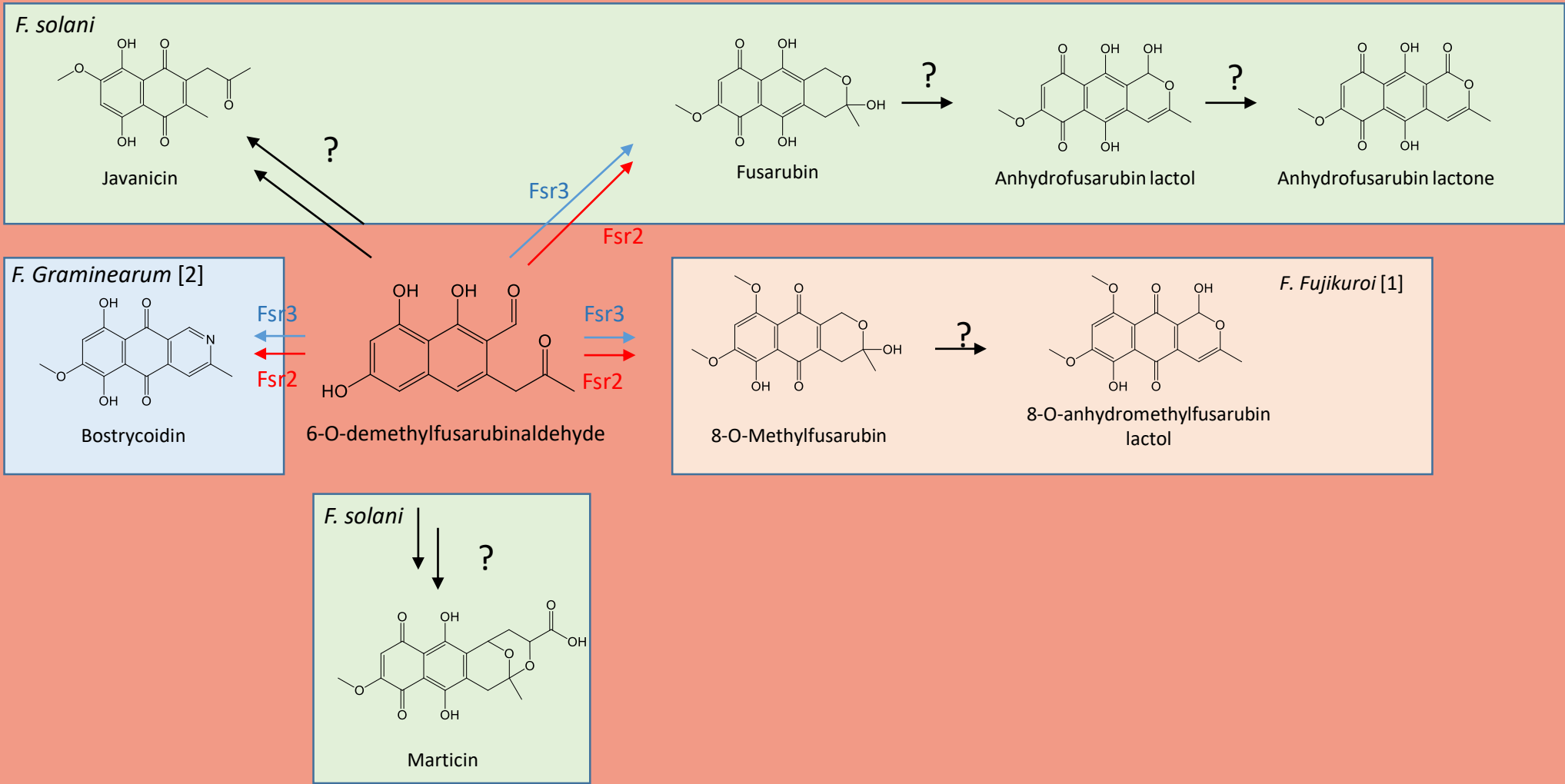


Mycelial pigmentation of *F. solani*

- PKS3 / *pgl1* / *fsr1* [1, 2] BGC present in all Fusaria [3] – the BGC is often associated with perithecial pigmentation in species outside the FSSC. Responsible for mycelial pigmentation in members of FSSC
- The *F. solani* PKS3 BGC shares seven genes with similar clusters in *F. graminearum* and *F. fujikuroi*. However, it differs from these clusters by containing several additional genes, some with predicted function related to secondary metabolism

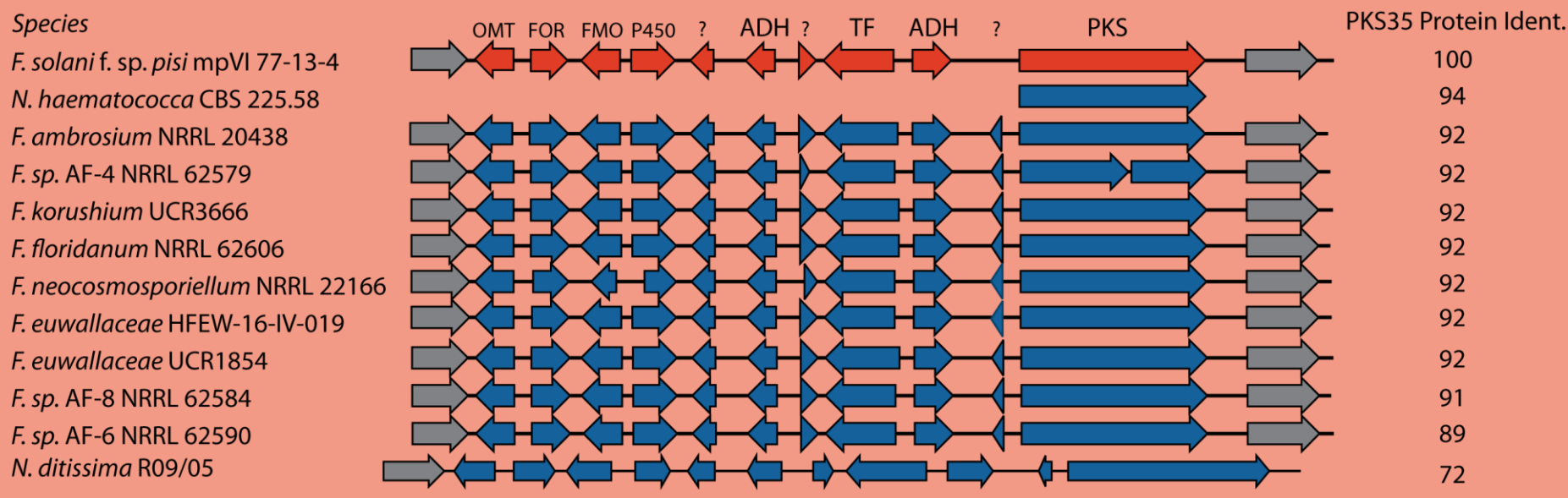


- The PKS3-related napthoquinones produced by members of the FSSC comprise fusarubin, javanicin, bostrycoidin, marticin, and derivatives of these molecules [5]

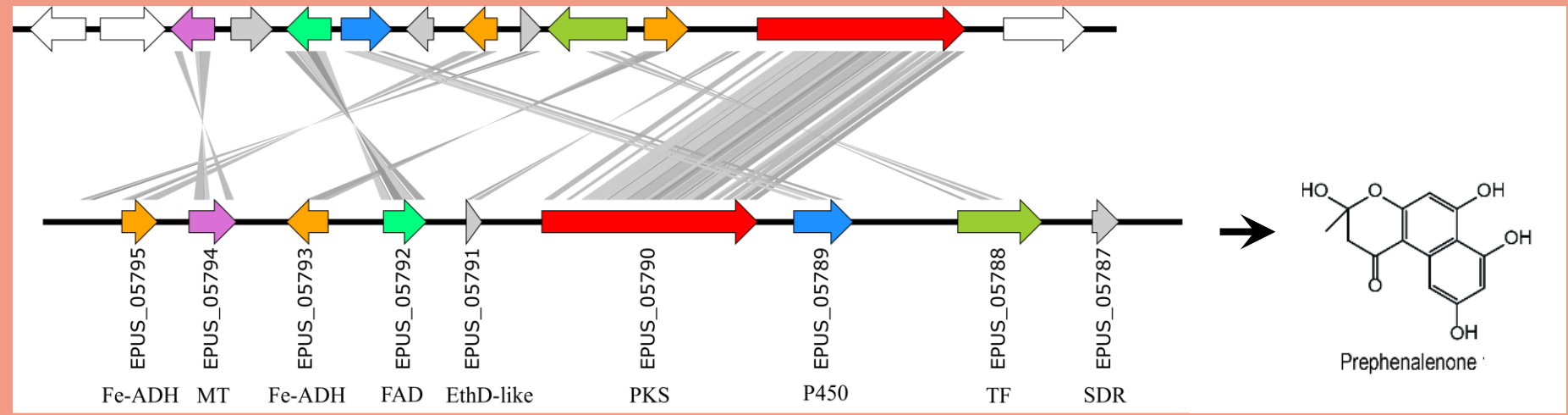


Perithecial pigmentation of *F. solani*

- The *pksN* /PKS35 BGC is associated with producing a red/orange perithecial pigment [4, 6] that is characteristic of FSSC species with known sexual reproduction [5].
- The PKS35 cluster might be unique to the FSSC, and has not been reported in other Fusaria [3]. 11 cluster genes are highly conserved in sequenced members of the FSSC [5, 7]

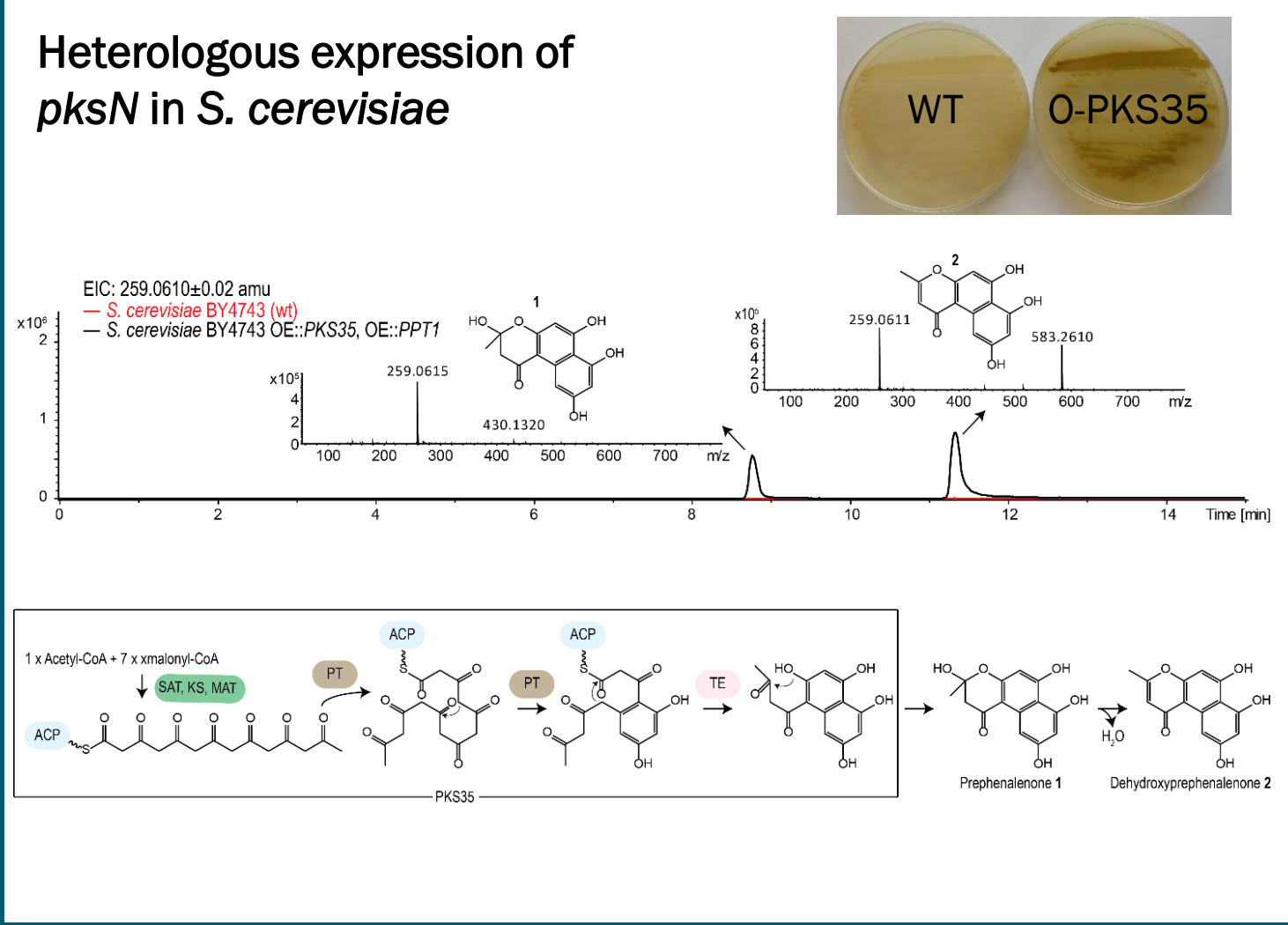


- A cluster in *Neonectria ditissima* shares high synteny to the PKS35 cluster. The genus is known for producing corymbiferan lactone E [8], a compound closely resembling herquinone[10]
- The PKS35 BGC shares eight ORFs with the lichen forming *Endocarpon pusillum* cluster PKS23. This cluster has previously been associated with the formation of prephenalene and dehydroxyphenalenone [9]

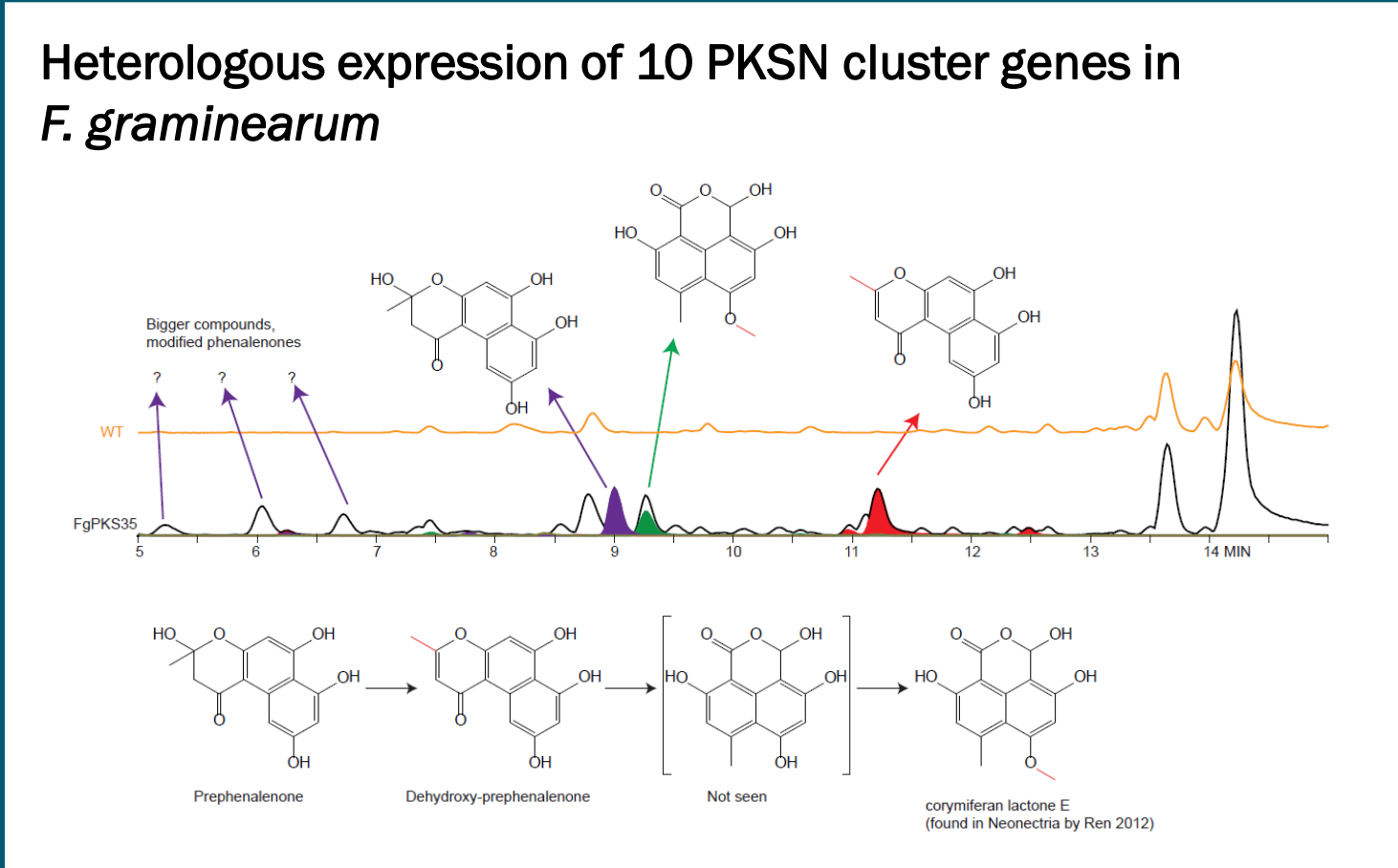


- Recently, a study on FSSC species *F. neocosmosporiellum* [6] reported high similarity to the herquinone producing *phn* BGC characterized in *P. herquei* [10]. The product initially released from the PKSN ortholog PhnA was identified as prephenalene.
- Phylogenetic analysis of PT domains from NR-PKSs grouped *F. solani pksN* together with *P. herquei phnA* in a clade of polyketides performing C4-C9 cyclization (not shown)

Heterologous expression of *pksN* in *S. cerevisiae*



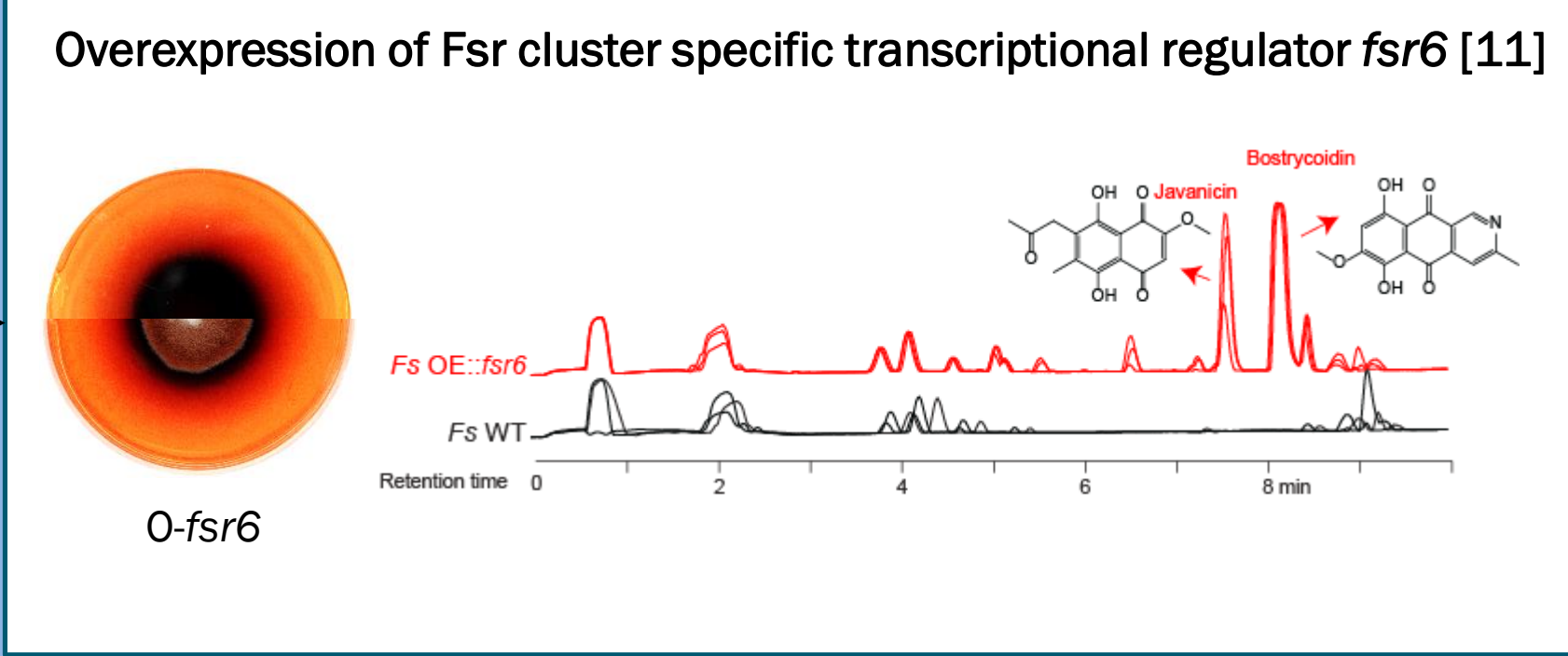
Heterologous expression of 10 PKS cluster genes in *F. graminearum*



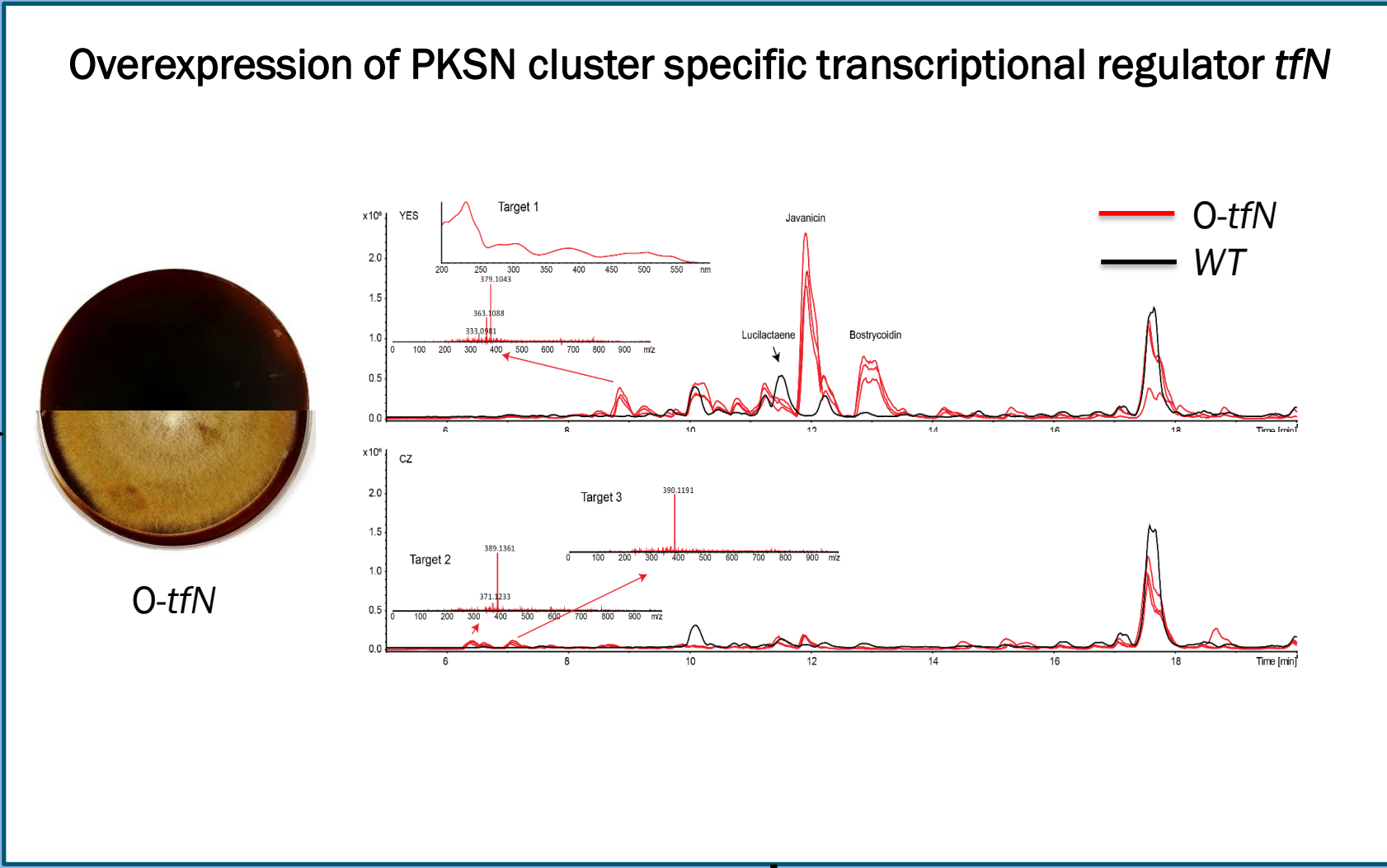
Concluding remarks on *F. solani* pigmentation

- The mycelial pigmentation is controlled by *fsr1* which releases 6-O-methylfusarubinaldehyde, the precursor for a wide range of coloured napthoquinones. The *F. solani* PKS3 BGC comprises several additional genes in comparison to that of other Fusaria. Meanwhile, additional PKS3-derived compounds are produced only in species within the FSSC e.g. javanicin.
- Perithecial pigmentation is driven by the conserved *pksN*. Nucleotide analysis suggests the initial release product is prephenalene. A mass fitting prephenalene was, together with larger masses, observed in overexpressing and heterologously expressing mutants. Curiously, an increase in PKS3-derived metabolites was observed when overexpressing the PKS35 intrinsic TF. This observation indicates the regulation of both clusters is somehow connected
- In this study we applied several strategies to map the products formed from the PKS35 BGC: Overexpression, gene deletion and heterologous expression. Concerning future studies, we will recommend applying a mixed methods approach to increase the likelihood of isolating novel compounds.

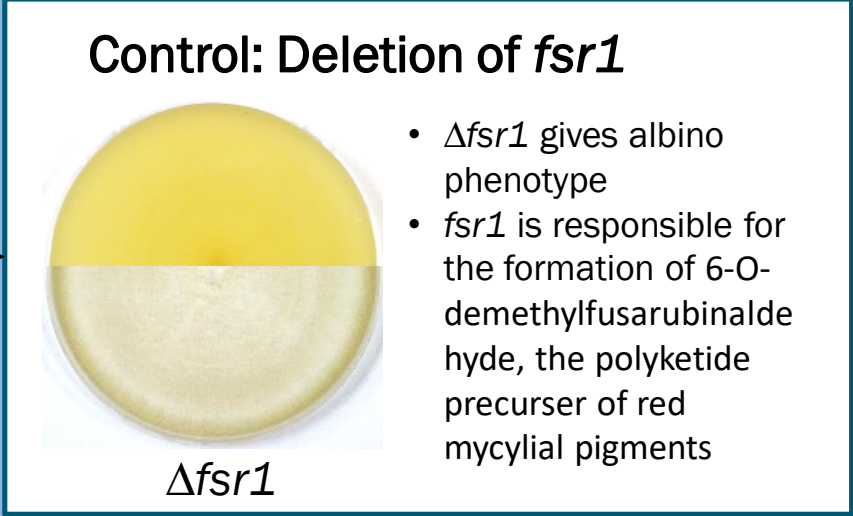
Overexpression of *Fsr* cluster specific transcriptional regulator *fsr6* [11]



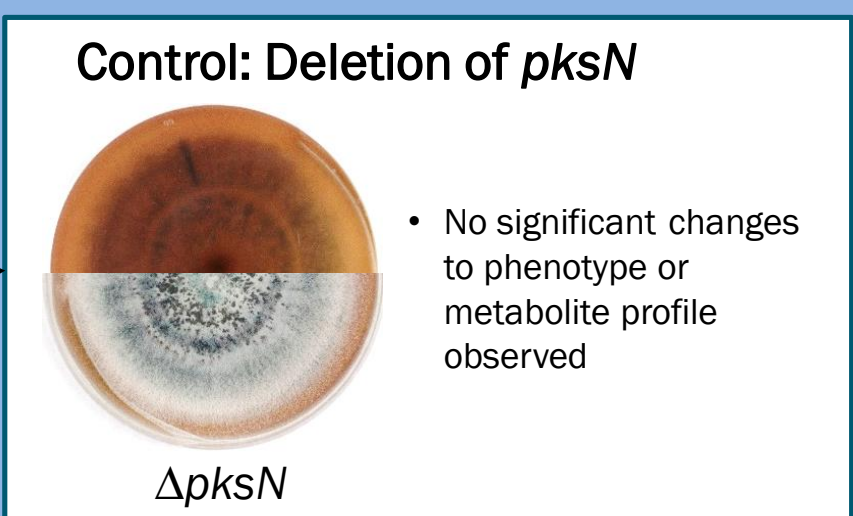
Overexpression of PKS cluster specific transcriptional regulator *tfN*



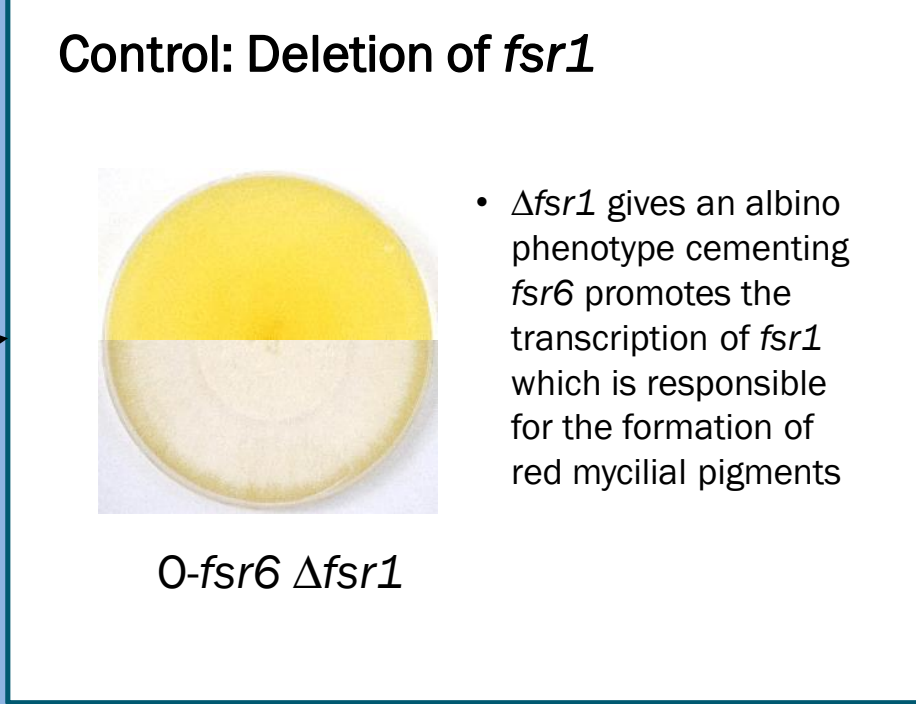
Control: Deletion of *fsr1*



Control: Deletion of *pksN*



Control: Deletion of *fsr1*



PKS35-related candidate compounds

Strain	[M+H] <sup>+</sup>	Formula	Putative compound
Sc PKS35	259.0615	C <sub>14</sub> H <sub>10</sub> O <sub>5</sub>	Dehydroxyphenalenone
	379.1043	C <sub>18</sub> H <sub>18</sub> O <sub>9</sub>	Candidate 1
Fs O-tfN	389.1361	C <sub>19</sub> H <sub>20</sub> N <sub>2</sub> O <sub>7</sub>	Candidate 2
	390.1191	C <sub>19</sub> H <sub>18</sub> NO <sub>8</sub>	Candidate 3
	417.1769	C <sub>22</sub> H <sub>26</sub> NO <sub>7</sub>	Candidate 4
Fg PKS35	261.0763	C <sub>14</sub> H <sub>12</sub> O <sub>5</sub>	Corymbiferan lactone E

Construction of double mutants enables detection of PKS35-related compounds

